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Distribution and taxonomy of the Italian clovers belonging to *Trifolium* sect. *Vesicastrum* subsect. *Mystillus* (Fabaceae)

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Abstract

In this paper, we focused our attention on the taxonomy and distribution in Italy of taxa belonging to *Trifolium* sect. *Vesicastrum* subsect. *Mystillus*. A short description of these closely related clovers, based on the study of fresh plant material, revision of herbarium specimens, and analysis of the relevant literature, is provided. Diagnostic features were highlighted by means of high-resolution digital images and, accordingly, an identification key is given. *Trifolium spumosum*, *T. vesiculosum*, *T. mutabile* and *T. setiferum* are reported to be distributed throughout Italy. We have updated the Italian distribution of the first three species by providing new occurrences from Tuscany, Umbria, Campania, Basilicata, and Calabria and by correcting previous references. The current presence of *T. multistriatum* in southern Italy is confirmed by our study, while *T. setiferum* must be excluded from the flora of Sicily. The study resulted in five new regional occurrences and 16 changes of presence status. Finally, intermediate forms between *T. vesiculosum*, *T. multistriatum* and *T. mutabile* were found both in living material and in the revised herbarium specimens.

Keywords

Central and southern Italy, clovers, identification key, new records, *Trifolium* subsect. *Mystillus*

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Introduction

The genus *Trifolium* L. is cosmopolitan, with about 255 species occurring mostly in the northern hemisphere (Zohary and Heller 1984, Gillett and Taylor 2001, Smýkal et al. 2015). According to Ellison et al. (2006) and Zohary and Heller (1984), the genus presumably originated in the eastern regions of the Mediterranean where the largest number and greatest variety of species are concentrated to date. In this area, it is represented by over 150 species, the richest country being Turkey (over 100 species), the poorest Egypt (22 species) (Euro+Med 2006 onwards). Italy holds 72 species (Conti et al. 2005) and 7 out of 8 sections that are typically recognized in the infrageneric classification of the genus *Trifolium* based on morphology (Zohary and Heller 1984, Greuter et al. 1989, Coulot and Rabaute 2013, George et al. 2013). Clovers are widely grown as green manure crops, pasture, livestock forage or silage; crop species have become extensively naturalized and are becoming increasingly widespread. At least 16 species of *Trifolium* are actively cultivated (Gillett and Taylor 2001), of which 10 are of considerable agricultural importance (Zohary and Heller 1984).

Based on the more recent classification of the genus, derived from molecular phylogenetic evidence (Ellison et al. 2006), the traditional *Trifolium* sect. *Mystillus* (C.Presl) Godr. is included in subgenus *Trifolium* as sect. *Vesicastrum* Ser. subsect. *Mystillus* (C.Presl) Coulot & Rebaute (Coulot and Rabaute 2013). As widely reported in the literature, all species classified in this subsection present a well-developed bracteolate flower, regarded as a primitive feature, with a persistent corolla, which assumes an important role in fruit dispersion. The fruiting calyx is symmetrically, more or less inflated or vesicular and multi-nerved, at least in the upper part (Gibelli and Belli 1892, Pignatti 1982, Zohary and Heller 1984).

There is a wealth of literature on the European *Trifolium* representatives. Zohary and Heller (1984) summarized the extensive taxonomical history of *Trifolium* by providing detailed descriptions and illustrations of all recognized species, although information regarding some species within the Italian territory was lacking (Scoppola and Lattanzi 2016). Other reports were made by Coombe (1968), Muñoz and Devesa (1988), Greuter et al. (1989), Muñoz Rodriguez (1992, 1995), and Euro+Med (2006 onwards).

Among the classical studies, the monograph by Gibelli and Belli (1892) of sect. *Trigantheum* Gibelli & Belli (= sect. *Mystillus* p.p.) is the most exhaustive concerning the Italian species; their delimitation of the species is noteworthy and proves their great knowledge of the genus (Zohary and Heller 1984, Scoppola and Lattanzi 2016). Other treatments were made by Bertoloni (1850), Fiori (1925), Zangheri (1976), Pignatti (1982) and Conti et al. (2005) (see Table 1).

Currently, *T. mutabile* Port., *T. setiferum* Boiss., *T. spumosum*, and *T. vesiculosum* have been reported within the Italian territory and regarded as distinct species, while *T. multistriatum* W.D.J.Koch has either been completely ignored (Pignatti 1982), considered as a synonym of *T. setiferum* (Greuter et al. 1989, Conti et al. 2005, Giardina et al. 2007), or even included in *T. vesiculosum* as a variety (Fiori 1925, Gavioli 1948, Zohary and Heller 1984, Coulot and Rabaute 2013) or as a subspecies (Gams 1923,

Table 1. Main taxonomical treatments of the *Trifolium* subsection *Mystillus* species in Italy.

	***T. multistriatum* W.D.J.Koch**	***T. mutabile* Port.**	***T. setiferum* Boiss.**	***T. spumosum* L.**	***T. vesiculosum* Savi**
Euro+Med (2006 onwards)		*T. mutabile* Port. (*T. leiocalycinum* Boiss. & Spruner)	*T. setiferum* Boiss. (*T. rumelicum* (Griseb.) Halacsy; *T. multistriatum* Koch) (provisional)	*T. spumosum* L.	*T. vesiculosum* Savi
Conti F et al. 2005		*T. mutabile* Port.	*T. setiferum* Boiss.	*T. spumosum* L.	*T. vesiculosum* Savi
Greuter W et al. 1989		*T. mutabile* Port. (*T. leiocalycinum* Boiss. & Spruner in Boiss., *T. vesiculosum* subsp. *mutabile* (Portenschl.) Ponert.)	? *T. setiferum* Boiss. (*T. multistriatum* Koch, *T. rumelicum* (Griseb.) Halacsy, *T. vesiculosum* subsp. *multistriatum* (Koch) Arcangeli	*T. spumosum* L.	*T. vesiculosum* Savi
Zohary M & Heller D 1984	*T. vesiculosum* Savi var. *rumelicum* Griseb. (*T. multistriatum* Koch)	*T. mutabile* Portenschl. (*T. leiocalycinum* Boiss., *T. paleaceum* Portenschl.)	*T. setiferum* Boiss.	*T. spumosum* L. (*T. apulum* Horst ex All.)	*T. vesiculosum* Savi var. *vesiculosum*
Pignatti S 1982		*T. mutabile* Portenschlag.		*T. spumosum* L.	*T. vesiculosum* Savi
Zangheri P 1976	*T. vesiculosum* subsp. *multistriatum* (Koch) Arcangeli (*T. setiferum* Boiss.)	*T. mutabile* Portenschl.		*T. spumosum* L.	*T. vesiculosum* Savi subsp. *vesiculosum*
Coombe DE 1968	*T. multistriatum* Koch (*T. setiferum* Boiss., *T. rumelicum* (Griseb.) Halacsy)	*T. mutabile* Portenschl. (*T. leiocalycinum* Boiss.)		*T. spumosum* L.	*T. vesiculosum* Savi
Fiori A 1925	*T. vesiculosum* Savi β *multistriatum* Koch	*T. vesiculosum* Savi γ *mutabile* Portschg.		*T. spumosum* L. (*T. apulum* All.)	*T. vesiculosum* Savi α typicum
Gibelli G & Belli S 1892	*T. multistriatum* Koch	*T. mutabile* Portenschlag. (*T. leiocalycinum* Boiss. & Sprunn.)	*T. setiferum* Boiss. (*T. vesiculosum* var. *rumelicum* Griseb.)	*T. spumosum* L. (incl. *T. apulum* All., *T. argutum* Russel.)	*T. vesiculosum* Savi
Bertoloni A 1850		*T. mutabile* Portenschl. (*T. multistriatum* Koch, *T. vesiculosum* Reich. non Sav., *T. setiferum* Boiss.)		*T. spumosum* L. (*T. apulum* All.)	*T. vesiculosum* Sav.

Zangheri 1976). Scoppola and Lattanzi (2016) have recently restored it as a distinct species, mostly based on features provided by Gibelli and Belli (1892).

The gaps in knowledge and doubts indicated in the reports over the years concerning the units of the group clearly denote their problematic identification. More specifically, 'Flora d'Italia' (Pignatti 1982), the floristic reference in use for over 30 years, does not deal comprehensively with this group, even when referring to the monograph by Gibelli and Belli (1892), and only provides an incomplete description of the species. Therefore, the Italian distribution and coenology, particularly of *T. spumosum*, *T. multistriatum* and *T. mutabile*, are still under debate.

This paper is part of a broader research aimed at updating the distributional range and taxonomy of the Italian clovers. In particular, the aim of this study was to provide new chorological data on *T.* subsect. *Mystillus*, in the light of the new findings from central and southern Italy, the recent re-evaluation of *T. multistriatum*, field surveys, revision of herbarium specimens, and literature analysis.

Material and methods

This study is based on analysis of relevant literature, including protologues, field surveys and examination of herbarium specimens kept in BEOU, CAT, CLU, E, FI, GE, JE, K, L, LEC, MPU, NAP, PAD, RO, TSM, UTV (acronyms according to Thiers 2016), including some relevant or original material (according to McNeill et al. 2012) for the studied names.

We collected flowering and fruiting samples of *Trifolium multistriatum*, *T. mutabile* and *T. vesiculosum* from May 2015 to July 2016 in several central and southern Italian regions (for the collection sites refer to the collected specimens). Geographical coordinates, when indicated, are in the form of Decimal Degrees (DD) as defined by the World Geodetic System of 1984 (WGS84). The study involved the acquisition of high-resolution digital images of floral details from fresh and dried material, useful for species identification and comparison. Morphological observations were done under a Leica M60 stereomicroscope, using a Leica IC80 HD Digital camera. The images were processed by means of the application LAS V-3.8. Plants from some of the sampled populations were cultivated in pots and seeds were stored for further studies.

The geographical arrangement of each species involved in this study has been updated. It refers to the Italian administrative regions and uses the following symbols, according to Conti et al. (2005): "+" occurring, "0" no longer recorded, "-" recorded in the past by mistake, "A" alien plant at regional level. The new records or changes of presence status at regional level are highlighted with "new" in brackets.

Results

Based on our observations of fresh and dry material and of several digital images, we found that the following characters are crucial for the identification of *Trifolium* sub-

sect. *Mystillus* species occurring in Italy: shape of flowering and fruiting heads, corolla/calyx ratio, shape of the calyx tube and teeth, consistency and thickness of the fruiting calyx, presence/absence of prominent longitudinal nerves and transverse veins in the fruiting calyx, and leaflets shape of the lower leaves.

The studied clovers are annual species with a spring-summer cycle, inhabiting grassy places among shrubs, roadsides, field margins and other dry fallow lands. *Trifolium spumosum* is the earliest species, flowering in April-May. It is easily recognizable by its smaller size, highly branched, prostrate or sub-erect stem, small flower heads, and corollas slightly protruding from the calyx. The vegetative parts of *T. multistriatum* do not differ from those of *T. vesiculosum* and *T. mutabile* in the case of vigorous plants and when grown in fertile soil. The latter species, more thermophilous and xerophilous, often appears with plants of very small size. All these species are glabrous, with many erect or ascending stems, rather long-petioled lower leaves, short-petioled to sub-sessile upper leaves, elliptical, acute, serrulate-dentate and mucronulate leaflets. They have many flower heads, which are terminal and axillary, globular to elongate in fruit (Figure 1), sustained by long or short, thick, peduncles; the floral bracts are lanceolate with prominent longitudinal nerves; the calyx is variable in shape among different species (Figure 2A–C); the corolla exerts from the calyx, it is white at anthesis then reddish to purple, persistent and then becoming scarious after flowering (Figure 1). The pod is membranous, long-beaked, generally 2-seeded; the seeds are granulate-verruculose, sub-globular to ovoid in shape and light brown or dark mottled (Figures 3–5). *Trifolium setiferum*, conversely, has rather short-petioled leaves, obovate-cuneate leaflets with a long spiny mucro at the apex, rather short peduncles, smaller heads, and subulate-setaceus calyx teeth, with a lanceolate base that is ciliolate.

The study resulted in five new regional occurrences and 16 changes of presence status.

Distribution and taxonomy

***Trifolium multistriatum* W.D.J.Koch, Syn. Fl. Germ. Helv., ed. 2. 190. 1843 [19-21 Jun 1843]**

Type (holotype indicated by Scoppola and Lattanzi 2016: 276). S. Andrea bey Triest, August 1840, *Tommasini* (L).

Description. Heads large; fruiting calyx ovoid with thick, longitudinal nerves all along, closed-spaced, transverse veins slightly visible; corolla > than twice as long as the calyx tube, seeds ovoid, light brown and dark mottled (Figures 1, 2B and 3).

Updated geographical distribution. Friuli Venezia-Giulia: 0A, Liguria: 0A, Campania: + (new), Basilicata: + (new), Calabria: + (new), Sicily: - (new).

Notes. The identity and autonomy of the name *Trifolium multistriatum* compared to the other species with whom it is closely related is discussed by Scoppola and Lattanzi (2016). The original material (L!) was recorded in northern Italy during the second half of the nineteenth century and later disappeared. Its occurrence might be

Figure 1. Flowering head of *Trifolium multistriatum* from Seminara (Reggio Calabria) (Photo by L. Bernardo, 1 June 2016).

due to the transport of seeds by ship solid ballasts collected at the port of departure during the commercial activities of the Austrian Lloyd shipping company in the mid- to late-1800s (Mack 2004). In BEOU, FI, PAD and TSM, there is evidence of the presence of the species at that time also in Dalmatia, Albania and Montenegro (Visiani 1852, Nyman 1878). Specimens at MPU were collected in 1831, 1851 and 1858 at Port-Juvénal, Hérault (southern France), where *T. multistriatum* (under the name *T. vesiculosum* var. *rumelicum* Griseb.) is considered as a historical alien species (Coulot and Rabaute 2013) as in Austria and Switzerland (Gams 1923, Zohary and Heller 1984). Even Fiori (1925) lists the species for Liguria as sub *T. vesiculosum* var. *multistriatum* W.D.J.Koch, writing: "È stato osservato avvent. … in Lig. ma a quanto pare vi è scomparso". According to the above, the report appears plausible.

The delimitation, status and distribution of *T. multistriatum*, with respect to *T. setiferum* and to *T. vesiculosum* var. *rumelicum*, is still under debate (see also Greuter et al. 1989, Euro+Med 2006, Scoppola and Lattanzi 2015, 2016). Here we confirm the

Figure 2. Comparison between mature calyces of *Trifolium* subsect. *Mystillus* species in Italy (bar = 1 mm). Left: *T. vesiculosum* (**A**) and *T. spumosum* (**A'**), middle *T. multistriatum* transitional (**B**) and typical (**B'**) forms, right *T. mutabile* s.str. (**C**) and *T. mutabile* var. *gussoneanum* (**C'**).

historical populations of Basilicata and Calabria (Fiori 1925, Gavioli 1948) and add a new locality in Campania. The presence in Campania at the foothills of the Vesuvius (RO!) was not confirmed either by Ricciardi et al. (1986) nor by our own research. However, the discovery in 2015 of a native population of the species in the Ofanto valley (Aquilonia, Avellino) has confirmed its occurrence in Campania. Gavioli (1948) collected the species in Basilicata in 1939 in the historical locality of Mt. Vulture, already known to Fiori (FI!). We also found it around the lakes of Monticchio and at San Michele (Rionero in Vulture), even at the edges of resting fields. We cannot exclude the presence of transitional forms towards *T. vesiculosum*, already assumed by Gibelli and Belli (1892) in this area (Figure 3). We also confirm the Calabrian historical localities where the species is locally abundant; some populations, still under study, show longitudinal calyx nerves that are more prominent and often in greater number and a less swollen calyx (Figure 2B'), which are not typical for *T. multistriatum* (Boissier 1872, Gibelli and Belli 1892, Hossain 1961, Zohary and Heller 1984). We also found transitional forms towards *T. mutabile* var. *gussoneanum* Gibelli & Belli, a variety already known for Sicily (Giardina et al. 2007; La Rosa 2011) and confirmed by our study in Calabria, that deserves further investigation.

The sole report of this species from Sicily (provisionally treated as *T. setiferum* by Greuter et al. 1989, Conti et al. 2005 and Giardina et al. 2007) is based on an old record "Castellammare, Todaro (sine indicatione nominis ac loci typici)...." in Lojacono (1909). Giardina et al. 2007 do not confirm its autonomy; in addition, Todaro's alleged sample, who reports the location Castellana, and not Castellammare (PAL!), is undoubtedly to be referred to *T. mutabile* var. *gussoneanum*. In the absence of clear references and specimens, we prefer not to confirm the presence of this species in Sicily.

Figure 3. *Trifolium multistriatum*, transitional forms (bar = 1 mm). Fresh flowers (24 June 2016) and seeds (5 August 2016) from plants collected in Rionero in Vulture (Potenza); detailed calyces from a specimen collected in the Ofanto valley (Aquilonia, Avellino) (CLU).

Collected specimens. Italy. Campania: Aquilonia, nei pressi del Fiume Ofanto, 40.963606°N. 15.557549°E, 339 m, 22 June 2016, *A. Scoppola* and *E. Lattanzi* (UTV); **Basilicata**: Rionero in Vulture, Laghi di Monticchio presso le rovine, 40.933504°N 15.610037°E, 670 m, 4 June 2016, *M. Pellegrino* (UTV); *ibidem*, nei pressi dell'Abbazia di S. Michele, 40.936284°N 15.615824°E, 740 m, 21 June 2016, *A. Scoppola* (UTV); *ibidem*, lungo la SS167 al km 12, 40.937079°N 15.593855°E, 708 m, 21 June 2016, *A. Scoppola* and *E. Lattanzi* (UTV). **Calabria**: Zagarise, SP Cuccuma-Mortilla nei pressi del ponte sul Torrente Ucria, 38.961982°E 16.709727°N, 24 June 2015, *L. Bernardo* and *P. Rizzo* (CLU); Tarsia, prov. Cosenza, poco oltre la diga, sulla strada che sale per Quercia tonda e Masseria Conte Giannone, 39.618701°N 16.314887°E, 100 m, 9 June 2016, *L. Bernardo* and *G. Maiorca* (CLU); Fiumefreddo Bruzio, prov. Cosenza, lungo la SP 45 nei pressi di Contrada Santa Serra, 39.229793°N 16.072064°E, 350 m, 20 June 2016, *A. Scoppola, L. Bernardo* and *E. Lattanzi* (CLU, UTV); *ibidem*, lungo la SP 45, fra le contrade Santa Serra e San Biase, 39.227341°N 16.076843°E, 410 m, 20 June 2016, *A. Scoppola, L. Bernardo* and *E. Lattanzi* (CLU, UTV); San Giovanni in Fiore, prov. Cosenza, Contrada Pietramela, lungo la SS108 per Savelli, 39.273356°N 16.720064°E, 958 m, 21 June 2016, *A. Scoppola, L. Bernardo* and *E. Lattanzi* (CLU, UTV); *ibidem*, Contrada Appendicane, lungo la SS108 per Savelli, 39.292576°N 16.744233°E, 803 m, 20 June 2016, *A. Scoppola, L. Bernardo* and *E. Lattanzi* (CLU, UTV); Varco San Mauro, prov. Cosenza, lungo la SS279, Rose, 39.419186°N 16.378297°E, 1221 m, 21 June 2016, *A. Scoppola, L. Bernardo* and *E. Lattanzi* (CLU, UTV); Seminara, prov. Reggio Calabria, lungo il sentiero Tracciolino poco più in basso dei Piani della Corona, 38.323697°N 15.837171°E, 450 m, 1 June 2016, *L. Bernardo* and *G. Maiorca* (CLU).

Trifolium mutabile Port., Enum. Pl. Dalmatia 16. 1824

Type (holotype indicated by Zohary and Heller 1984: 283). In ins. Lissa, in campo grande 4 (Plate 12, Figure 1 in Portenschlag-Ledermayer 1824).

Description. Heads large, fruiting calyx tubular, longitudinal nerves barely visible in the distal portion; corolla > than twice as long as the calyx tube, seeds irregularly ovoid, light brown (Figures 2C and 4).

Updated geographical distribution. Liguria: 0A, Tuscany: +A (new), Umbria: +A (new), Lazio: +A (new), Campania: 0 (new), Apulia: +, Basilicata: 0 (new), Calabria: +, Sicily: +.

Notes. Species endemic to the Mediterranean area (Greuter et al. 1989), which was described for Dalmatia. In the wild it is widespread in the western Balkans (Dalmatia, Greece, Albania) and in southern Italy, from Campania to Sicily (Euro+Med 2006 onwards, Conti et al. 2005), where it is represented by *T. mutabile* var. *gussoneanum* (Figure 2C'), endemic to southern Italy "...Varietas haec T. mutabilem cum T. multistriatum ideo conjungit." (Gibelli and Belli 1892). The Sicilian authors propose to rank it as subspecies (Brullo in La Rosa 2011). The presence of *T. mutabile* in the Iberian Peninsula is most likely occasional (Muñoz and Devesa 1988, Greuter et al. 1989).

Here, we report it for the first time in central Italy both in the countryside south of Manciano (Grosseto), where it was found to be abundant in fallow and resting fields as well as wood edges, and in the outskirts of Castel Giorgio and of Orvieto (Terni), not far from the Lazio border, where it was observed in small populations of fallow fields and roadsides. In both stations, together with the one recently reported in Lazio (Lopez Tirado et al. 2015), the species is believed to be a naturalized alien. In central Italy, it is used as forage in multiple crops with *T. squarrosum* L. or *T. incarnatum* L. in arid localities (e.g. San Lazzaro, Viterbo) where it escapes from cultivation. As a non-native species it was reported in Liguria by Fiori and Paoletti (1900) but never confirmed.

Trifolium mutabile is known in Campania (Conti et al. 2005), where it was reported from Roccamonfina (Caserta) by Croce et al. (2008), although samples are not available. Here we repeatedly looked for it in the outskirts of the Masseria S. Anna but were only able to find *T. vesiculosum*. Nevertheless, two different historical samples collected in the Campi Flegrei area by Nicola Terracciano (NAP!) and near the Lake of Agnano by Heldreich in 1840 (FI!, revised by Gibelli and Belli as var. *gussoneanum*) belong to this species. Thus, the presence of the species in Campania deserves to be confirmed. It is considered as occurring in Apulia by Conti et al. (2005) based on Pignatti (1982) and Fiori (1925, RO!) and by Mele et al. (2006); it was also collected by P. Medagli (LEC!).

Finally, the current presence of *T. mutabile* in Basilicata (Conti et al 2005) needs confirmation, since it is only based on the specimens reported by Gavioli (1948) as a variety of *T. vesiculosum* (kept in FI! and revised by us as *T. mutabile* var. *gussoneanum*), dating back to the 1930s.

Collected specimens. Italy. **Tuscany**: Manciano, strada sterrata verso il Pod. Il Pelargone 42.538296°N 11.543912°E, 253 m, 26 May 2016, *A. Scoppola* (UTV); **Umbria**: Orvieto, SR71 ter Umbro-Casentinese al km 14.6, 541 m, 42.662446°N

Figure 4. *Trifolium mutabile* collected in Viterbo (bar = 1 mm). Flowers (15 May 2015), seeds (15 July 2016).

12.031857°E, 25 June 2016, *A. Scoppola* (UTV); Castel Giorgio, Loc. Casa Perazza, 42.680235°N 11.979294°E, 580 m, 25 June 2016, *A. Nizzoli* and *A. Scoppola* (UTV). **Lazio**: Viterbo, strada San Lazzaro, 42.443156°N 12.078430°E, 311 m, 14 May 2016, *A. Scoppola* (UTV). **Calabria**: (***T. mutabile* var. *gussoneanum*** Gibelli & Belli) – Nocera Terinese, prov. Catanzaro, A3 direzione Nord, fra la galleria e il viadotto Ogliastro, fra gli svincoli di Falerna e San Mango d'Aquino, 39.042581°N 16.145387°E, 162 m, 1 June 2016, *L. Bernardo* and *G. Maiorca* (CLU).

Trifolium setiferum Boiss., Diagn. Pl. Orient. ser. 1, 2: 32. 1843 [Mar 1843]

Type (holotype indicated by Zohary and Heller 1984: 280): (Prov. Izmir) Montagne de Jenidje, May 1842, *Boissier* (G).

Description. Heads small; fruiting calyx inflated, calyx teeth with ciliolate base; longitudinal veins numerous; corolla longer than calyx. Seeds brown (Zohary and Heller 1984).

Updated geographical distribution. Calabria: - (new), Sicily: - (new).

Notes. Described from the Province of Izmir in Turkey, it is probably an eastern Mediterranean endemic naturally occurring only in Turkey and Greece, although many sources suggest it has a wider distribution based on its assumptive synonymy with *T. rumelicum* and/or *T. multistriatum*. We agree with Boissier (1872) and Zohary and Heller (1984) who separate this species from the latter based on the smaller size of the flower heads, the different shape of the leaves, the inflated calyces characterized by bristles at the base of the teeth. Pignatti (1982) does not report the species at all in Italy. All citations for Italy in fact have to be referred to *T. multistriatum* (Zangheri 1976, Greuter et al. 1989, Scoppola and Lattanzi, 2016). The species is only reported for Calabria by Zohary and Heller (1984), who probably refer to the same sample of *T. multistriatum* by Fiori of 1899 (FI, RO!) also cited under the name *T. vesiculosum*

var. *rumelicum*. Greuter et al. (1989) report it as putative species for the mainland and Sicily also according to the synonymy with *T. vesiculosum* subsp. *multistriatum* (W.D.J.Koch) Arcang., indicated by Zangheri (1976), who reported the entity in southern Italy and Sicily. Accordingly, no evidence was found to confirm its presence on the Italian territory.

Trifolium spumosum L. Sp. Pl. 2: 771. 1753 [1 May 1753]

Type (lectotype designated by Zohary and Heller 1984: 274): In Gallia, Italia, Apulia. Hort. Clifford, 373, 7 (BM).

Description. Flowering heads small (15-20 mm); fruiting calyx vesicular-turbinate, netted-nerved; corolla short, slightly protruding from the calyx (Figure 2A'), seed ovoid, light brown.

Updated geographical distribution. Liguria: 0 (new), Tuscany: 0 (new), Marche: 0, Lazio: +, Abruzzo: 0 (new), Campania: 0 (new), Apulia: 0 (new), Basilicata: 0 (new), Calabria: +, Sicily: +, Sardinia: +.

Notes. It is a widespread steno-Mediterranean thermophilous and xerophilous species, especially found in dry uncultivated lands of coastal areas. It was described on the basis of collections made in Italy and France. Based on our data, the distribution in Italy is very fragmented and in decline, especially in the central regions. A similar depletion has been reported in southern France where the species is classified as naturalized over most of the territory (Coulot and Rabaute 2013). It is quite widespread in Sicily (Giardina et al. 2007, La Rosa 2011), where it mostly grows in arid uncultivated hilly and mountain areas. However, its current presence in the Italian southern regions, especially in Campania and Apulia, has probably been underestimated due to confusion with other taxa (e.g. *T. vesiculosum*, *T. resupinatum* L.).

Peccenini (2007) cites the report by Bertoloni (1850) "ex Liguria orientali in litore Clavarensi a Turio; Genua all'Acquasola a Vincentio" and by Fiori (1925) for Liguria; moreover her recent report in the outskirts of Sarzana (FI!, GE; Peccenini 2007) must be corrected, as the sample actually refers to *T. resupinatum*. Thus, we record the species as requiring confirmation in Liguria. Concerning Tuscany, no herbarium material can confirm the historical records of this species between Grosseto and Castiglione della Pescaia (Savi in Caruel 1862, Pignatti 1982, Selvi 2010), but several specimens from the Elba, Giglio, and Pianosa islands (FI!) confirm its presence in the past. Also in need of confirmation is its occurrence in Marche, where it was collected in Senigallia (Ancona) in 1876 (FI! as *T. vesiculosum*; Gubellini et al. 2014). The occurrence of the species in Abruzzo (Conti 1998) is by Zodda (1954) and by Crugnola (1894, 1900) who refers to a sample by Petrilli from the Mavone valley (Valle del Vomano, Teramo); we have been unable to trace it. The occurrence in Campania and Basilicata (Conti et al. 2005) is only based on historical records from the outskirts of Avellino, "Ad Aiello del Sabato" (Milani 1890, Casali 1901, Trotter 1905) and Caserta at S. Silvestro (Terracciano 1872) and on a gathering from an unknown locality by Tenore (NAP; A. Santangelo, pers. com.).

The Apulian material dating back to the 1970s, deposited in PAD and labeled as *T. spumosum*, actually refers to *T. resupinatum*. *Trifolium spumosum* has been reported from Apulia by Conti et al. (2005) based on these gatherings and on Bertoloni (1850), who cited a specimen collected in Foggia by Gussone (NAP!). The species is also reported in Salento by Bruni (1857) and Marinosci (1870), confirmed by Mele et al. (2006) and - for the Province of Brindisi - by Tomaselli et al. (2010). As no recent samples were found either in LEC or BI (P. Medagli and V. Tomaselli, pers. com.), we still consider the species in need of confirmation for the current Apulian flora. The presence in Lazio, Calabria, and Sardinia (Conti et al. 2005) is confirmed.

Trifolium vesiculosum Savi, Fl. Pis. 2: 165. 1798 (as '*T. vessiculosum*')

Type ('lectotype' designated by Zohary and Heller 1984: 276): Clairières de bois, Pisa, Juillet, *P. Savi* (M).

Description. Flowering heads large; fruiting calyx vesicular, turbinate, shining, netted-nerved, calyx teeth with base not ciliolate; corolla longer than calyx, seeds subglobular, light brown (Figures 2A and 5).

Updated geographical distribution. Tuscany: +, Umbria: +, Lazio: +, Abruzzo: +, Campania: +, Apulia: 0 (new), Basilicata: 0 (new), Calabria: 0 (new), Sicily: 0 (new), Sardinia: +.

Notes. It is a Mediterranean species growing from southern to central Europe on fertile, well-drained soils. This clover is cultivated on a large scale for forage and has a good ability of self-sowing because of the high production of hard seeds (Gillett and Taylor 2001, Coulot and Rabaute 2013). It was repeatedly cited by Italian botanists for central and southern Italy. However, its presence has probably been overestimated mainly in the past due either to the different taxonomical treatments of closely-related species or to confusion among them. In particular, we cannot confirm the species in Apulia, although it is indicated by Conti et al. (2005). Tenore (1835–1838) reports it for Gargano where it is not confirmed by Licht (2008). Additional data come from Béguinot (in Fenaroli 1970) but we were not able to find herbarium samples for the mentioned locality (Bosco Sfilzi). Concerning Basilicata, the indications from Balvano (Barbazita) and Muro Lucano (N. Terracciano), as well as the samples by Trotter (Gavioli 1848) could not be verified till now. Gibelli and Belli (1892) identify a *T. vesiculosum* var. *stenodictyon* Gibelli & Belli from Mt. Vulture, based on samples collected by Terracciano and Gussone, corresponding to intermediate forms (hybrids?) between *T. vesiculosum* and *T. multistriatum*, but *T. vesiculosum* s.str. is not confirmed either from the literature or by recent gatherings.

This species is considered as occurring in Calabria by Conti et al. (2005), based on Zohary and Heller (1984), who assigned a specimen of *T. multistriatum* from Fiumefreddo Bruzio (A. Fiori, 1899, FI!) to *T. vesiculosum* var. *rumelicum*. Moreover, for the same region there are also some old reports (Porta 1879, Macchiati 1884) dating back

Figure 5. *Trifolium vesiculosum* collected in Viterbo (bar = 1 mm). Flowers (4 July 2016), pod and seeds (2 August 2016).

to a time when *T. mutabile* and *T. multistriatum*, both currently occurring in Calabria, were considered as varieties of *T. vesiculosum*. Thus, the latter species is here recorded as requiring confirmation.

The presence of the species by Conti et al. (2005) in Sicily derives from Brullo (1982), who cites it in some phytosociological relevés. It is also reported by Gussone (1843) and by Giardina et al. (2007), but questioned by La Rosa (2011) and by Giardina et al. (2007), who quote many references and state "... all localities of Southern Sicily are doubtful due perhaps to the limited identification made by the authors to the rank of species to *T. mutabile* (*T. vesiculosum* Savi subsp. *mutabile* Portenschl.". To the latter species we attribute the Sicilian reports of *T. vesiculosum*. However, the sample in CAT (http://www.dipbot.unict.it/herbarium/foto/06/006028.jpg) from Cefalù, clearly refers to *T. vesiculosum* and confirms the presence of the species, at least in the past.

Collected specimens. Italy. **Tuscany**: Manciano, Monti di Castro Loc. Campigliola, 42.526919°N 11.519618°E, 27 June 2015, *A. Scoppola* (UTV). **Umbria**: Castel Giorgio, lungo variante via Parma 42.712320°N 11.979294°E, 543 m, 25 June 2016, *A. Nizzoli* and *A. Scoppola* (UTV); Orvieto, Loc. Canonica, 42.700492°N 12.050351°E, 460 m, 25 June 2016, *A. Nizzoli* and *A. Scoppola* (UTV); **Lazio**: S. Martino al Cimino, Viterbo, Loc. Chiesa Nuova, 42.366113°N 12.120279°E, 517 m, 4 Jul 2016, *A. Scoppola* (UTV). **Campania**: Sessa Aurunca, Parco regionale Roccamonfina, lungo strada per S. Maria Valogno, 41.303914°N 13.894509°E, 60 m, 3 June 2016, *A. Scoppola* and *E. Lattanzi* (UTV); *ibidem*, lungo SS430 nei pressi di Masseria S. Anna, 26 m, 41.309700°N 13.896533°E, 3 June 2016, *A. Scoppola* (UTV); *ibidem*, SS430 incrocio strada per S. Carlo, 41.319240°N 13.890250°E, 27 m, 3 June 2016, *E. Lattanzi* (RO); Masseria S. Anna, Parco di Roccamonfina, 41.311930°N 13.897133°E, 42 m, 5 June 2016, *A. Nizzoli* (UTV).

Identification key to the species of *Trifolium* subsect. *Mystillus* in Italy

1 Fruiting calyx inflated, more or less vesicular, turbinate and shining (Figure 2A), netted-nerved, with longitudinal and transverse veins all along; the largest heads with very close flowers **2**

– Fruiting calyx tubular or oblong to ovate, not or slightly inflated, opaque and rigid, never vesicular (Figure 2C), longitudinal nerves more or less thick, transverse veins absent or barely visible in the distal tube portion and teeth (or in dry plants); heads elongating in fruit, with less dense flowers **3**

2 Flowering heads small (15–20 mm), ovoid; bracts broad and shorter than the calyx tube; standard slightly protruding from the calyx (< than twice the tube length), straight after anthesis; leaflets obovate to rhombic, truncate to retuse at apex ..... ***T. spumosum***

– Flowering heads larger, dense, bracts narrower and as long as the calyx tube; standard longer than calyx (≥ than twice the tube length), deflexed after anthesis; leaflets elliptic to obovate, acute, mucronulate **4**

3 Calyx tube oblong, cylindrical, not narrowed at throat in fruit, with longitudinal veins slightly visible only in the distal portion (below the teeth) ..... ***T. mutabile***

– Calyx tube ovoid, slightly narrowed at throat in fruit, with many longitudinal, thick nerves all along, generally very closed spaced; transverse veins not visible or slightly, thick and forming very small and regular meshes ..... ***T. multistriatum***

4 Calyx teeth lanceolate-subulate with base not ciliolate, fruiting tube vesicular, turbinate, shining, clearly netted-nerved, with wide meshes; terminal heads larges (25–45 × 20–35mm), globular to ovate, elongating in fruit; leaflets large, elliptic, acute or mucronulate ..... ***T. vesiculosum***

– Calyx teeth subulate-setaceus, with base ciliolate; fruiting tube inflated, white, thin walled, not vesicular; longitudinal veins numerous, transverse ones slightly visible; terminal and axillary heads smaller (20–25 × 15–20), ovate-globular to ovoid in fruit; leaflets obovate-cuneate with a terminal spiny mucro ..... ***T. setiferum***

Concluding remarks

Our study has shown that knowledge on the distribution of clovers belonging to *Trifolium* subsect. *Mystillus* in Italy is still largely incomplete. The revision of herbarium samples, the study of all literature data as well as field collections and observations have highlighted that, in many Italian regions, *T. vesiculosum* and *T. spumosum* have been reported in the past by mistake or through lack of recent reports; *T. setiferum*, instead, must be excluded from the national flora (Figure 6). On the other hand, both *T. mutabile* and *T. multistriatum* should be added to the flora of several Italian administrative regions.

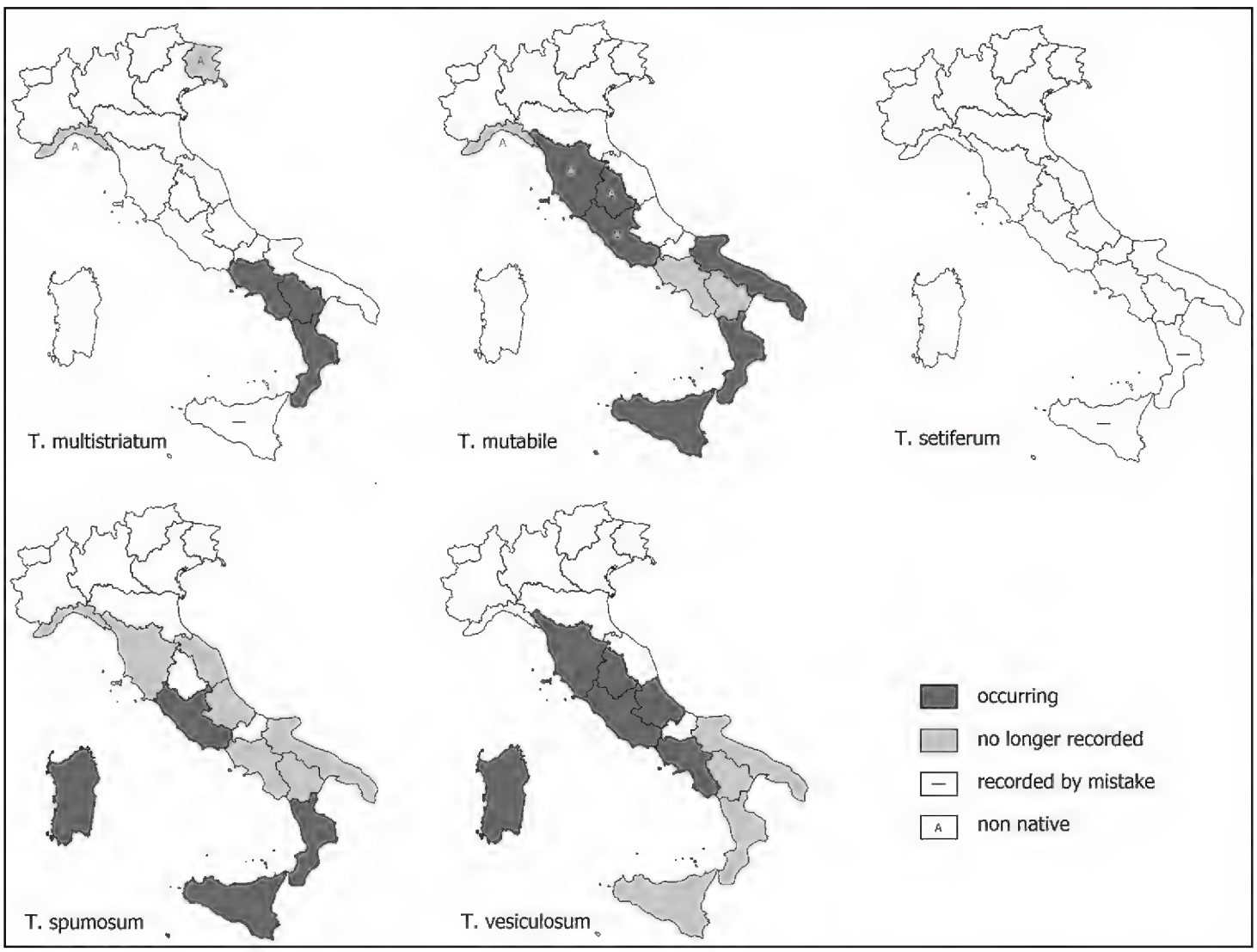

Figure 6. Regional distribution maps of the concerned species.

The delimitation and status of *T. multistriatum*, with respect to *T. vesiculosum* var. *rumelicum*, is to be considered problematic (see also Coombe 1968, Greuter et al. 1989, Conti et al. 2005, Euro+Med 2006 onwards, Scoppola and Lattanzi 2016). The fresh material examined, in fact, showed a high similarity both with the original material from Trieste and also with the samples of *T. vesiculosum* var. *rumelicum* present in FI, RO, BEOU. Halácsy (1901) elevated *T. vesiculosum* var. *rumelicum* to species rank explaining that *T. rumelicum* (Griseb.) Halácsy (1901: 399) "... differt a T. multistriatum calyce fructifero breviore, turbinato, inflato, inter nervos obscure rugulosoreticulato, laciniis latioribus brevioribus". Some of these characters are also present in the Italian populations (Figures 2B and 3). We do not know the type (in GOET, Zohary 1970) of this variety but in FI there are samples from Bulgaria, Macedonia and Greece that are useful for comparison.

Based on a careful examination of the floral details of the relevant material of *T. multistriatum* in PAD, and TSM and according to its alleged status as occasional historical alien, at the present state of our knowledge, we can agree with other authors in classifying the two names as heterotypic synonyms, confirming the rank of species to the entity *Trifolium multistriatum*. This unit, in fact, has long been neglected or considered of uncertain location and may result from repeated episodes of hybridization, as we can hypothesize for the Balkan Peninsula. In southern Italy, this could have occurred

from sympatric populations of *T. vesiculosum* and *T. mutabile*. These episodes may have occurred repeatedly, resulting in forms converging into each other, thus in that continuum of morphological traits of the calyx (Figure 2) or vegetative apparatus that have been highlighted in this study both in fresh and dry specimens of the populations from Campania, Basilicata and Calabria. In particular, in Calabria the populations of *T. multistriatum* of the historical localities of Fiumefreddo (A. Fiori, RO) and San Giovanni in Fiore (G. Lopez, RO) appear to be solid and well characterized, also from a palynological point of view (A. Scoppola and collaborators, in preparation). Conversely, the population of Varco San Mauro appears to be a transitional form to *T. vesiculosum*, based on calyx and corolla morphology, poor fertility and color of the seeds. S. Gentile collected this unit in resting fields in the nearby Ceraso valley already in 1960, a time when large areas of the Sila massif were intensively cultivated with cereals and forage.

Finally, *T. mutabile* var. *gussoneanum* should certainly be re-evaluated, since it is widespread and well characterized at least in Sicily, where neither *T. vesiculosum* nor *T. multistriatum* have been confirmed; already in Calabria it appears to be more variable and with transitional forms to *T. vesiculosum* s.str. or to *T. multistriatum* and requires confirmation in Basilicata and Campania.

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Selected specimens examined

Trifolium multistriatum W.D.J.Koch – **ITALY. Friuli Venezia Giulia**: Trieste, S. Andrea, August 1840, *M. Tommasini* (L n. 1997850 digital image!); Trieste, immunizione di Campo Marzio, 29 Jul 1840, *M. Tommasini* (TSM!); S. Andrea di Trieste, sine die, *M. Tommasini* (PAD!); **Campania**: Vesuvio all'Eremita, sine die, *G.A. Pasquale* (RO! under the name *T. vesiculosum* Savi); **Basilicata**: In Vulturis herbosis arenosis, June 1859, *N. Terracciano* (FI! under the name *T. mutabile* Port.); Rionero in Vulture, 700 m, 18 July 1918, *A. Fiori* (FI!); Rionero, ad pendices montis S. Michele prope lacum, 2 July 1939, *O. Gavioli* (FI!); **Calabria**: Sila, S. Giovanni in Fiore, 23 June 1913, *G. Lopez* (RO!); Calabria occidentale presso Fiumefreddo, 12 June 1899, *A. Fiori* (FI!, RO!); Sila, Valle Ceraso, 1300 m, 21 July 1960, *S. Gentile* (GE! under the name *T. vesiculosum* Savi); Sila Greca, Rossano, Bosco del Patire, 16 May 2009, *L. Bernardo* (CLU!), Aiello Calabro, Loc. Vallone presso Azienda Agrituristica Fargani, 498 m, 7 July 2005, *V. Pignataro* (CLU!). **CROATIA. Split-Dalmatia**: In histricis circa Traù, sine die, *A. Andrich* (PAD!). **ALBANIA**. Vallona: In cultis ad Pogdania prope Suernec, 29 June 1894, *A. Baldacci* (FI!). **BULGARIA. Haskovo**: Bogomil, 4 June 2013, *P. Coulot* (photo in Coulot and Rabaute 2013, under the name *T. vesiculosum* var. *rumelicum* Griseb.). **Southern Rhodope Mountains**: prope pag. Menkova, 16 June 1871, *V. Janka* (FI!); **SERBIA. Pčinja**: Vranje, 1878, *J. Pančić* (BEOU digital image!). Nišava: Bukovanj okrug Aleksinački, 1852, *J. Pančić* (BEOU digital image!). **MONTENEGRO. Danilovgrad**: Zeta pr. Sp(už), sine die, *J. Pančić* (BEOU digital image!). **GREECE. Thessalia**: In planitia pelargonica ad rad. M. Olympi, Thessaliae, 5 August 1851, *T. Heldreich* (FI!, under the name *T. vesiculosum* Savi). **FRANCE. Hérault**: Montpellier, Port Juvenal, May 1931, s.c. (MPU).

Trifolium mutabile Port. – **ITALY. Lazio**: Bomarzo, R.N. Monte Casoli, 184 m, 12 June 2009, *A. Scarfone* (UTV! under the name *T. vesiculosum* Savi); Viterbo, nei pressi del cimitero, 324 m, 15 May 2015, *J. López Tirado* (UTV!, FI!); **Apulia**: Spinazzola, in pascuis Murge dictis, 500 m, 9 June 1913, *A. Fiori* (RO!); Loc. Marangi, Lecce, 28 July 2015, *P. Medagli* (LEC digital image!); ***T. mutabile* var. *gussoneanum*** Gibelli & Belli – **Campania**: In aridis agri Neapolitani (al lago d'Agnano), September 1840, *Th. De Heldreich* (FI! under the name *T. vesiculosum* Savi); Campi Flegrei**,** Agnano, July 1906, *N. Terracciano* (NAP!); **Basilicata**: Potenza, Macchia Romana, 820 m, 12 July 1937, *O. Gavioli* (FI! under the name *T. vesiculosum* var. *mutabile*); Potenza, Loco S. Maria vocato, 800 m, 15 July 1937, *O. Gavioli* (FI! under the name *T. vesiculosum* var. *mutabile*). **Calabria**: Sersale, contrada Cipino Sottana, strada per Melissaro, 680 m, 5 June 2008, *L. Bernardo* (CLU!). **Sicily**: Castellana, sine die, s.c. (PAL! under the name *Trifolium*); Sughereta di Caronie, 4 July 1983, *S. Brullo* (CAT under the name *T. mutabile* Portensch. subsp. *gussoneanum*, digital image!); Abitato di S. Gregorio di Catania, 25 May 2003, *R. Galesi* (CAT, digital image!); Monte Scuderi, Peloritani, 29 May 2007, *S. Brullo*, *C. Ronsisvalle* and *S.Sciandrello* (CAT, digital image!).

Trifolium setiferum Boiss. – **TURKEY. Anatolia**: Caria, 1843, *C. Pinard* (RO!); Ephesus, sine die, *E. Boissier* (JE digital image!). **Manisa**: Valles Mesogis et Tmoli, July 1842, *E. Boissier* (FI!, K digital image!); **Izmir**: Mesogis, inter Dervent et Alaşehir, June 1842, *E. Boissier* (E digital image!).

Trifolium spumosum L. – **ITALY. Tuscany**: Elba**,** Rio al Padreterno, 1870, *E. Marcucci* (FI!); Isola del Giglio, 1898, *G. Doria* (FI!); Pianosa, 1909, S. Sommier (FI!); **Marche**: Senigallia, August 1876, *H. Ricci* (FI!); **Lazio**: Roma, Scalo Ostiense, 3 May 1951, *A. Cacciato* (RO!); ibidem, via Laurentina, 22 April 2006, *E. Lattanzi* (RO!); **Apulia**: Foggia, 16 May 1840, *G. Gussone* (NAP!); **Calabria**: Spezzano Albanese, 340 m, 20 April 1999, *L. Bernardo* (CLU!); Faudano, nei pressi del fiume Crati, S. Sofia d'Epiro, 70 m, 20 May 2006, *L. Bernardo* (CLU!); **Sardinia**: Dorgali, 1980, *P.V. Arrigoni* (FI!); Condogianos, Saccargia, 17 May 2002, *E. Lattanzi* (UTV!). **Sicily**: Niscemi, Arcia, Case Coloniche, 18 April 1990, *R. Galesi* (CAT digital image!); Modica, 13 April 1979, *S. Brullo* (CAT digital image!); Pantelleria, dietro l'isola, 20 April 1975, *S. Brullo* (CAT digital image!).

Trifolium vesiculosum Savi – **ITALY. Lazio**: Viterbo, Poggio Pelagi, 600 m, 25 July 1997, *C. Caporali* (UTV!); Caprarola, scendendo al Lago di Vico dalla strada Cimina, 18 July 1988, *A. Scoppola* (UTV!); Roma, Castelporziano, 15 June 2014, *E. Lattanzi* (RO!); Nettuno, Poligono militare, 11 July 2007, *G. Filibeck* and *E. Lattanzi* (UTV!); Marino, Monte Cavo, 12 June 2000, *M. Pellegrini* (UTV!); Sabaudia, Parco del Circeo, 31 May 1983, *A. Scoppola* (UTV!); **Abruzzo**: Oricola, Piana del Cavaliere in loc. Prata Lunghe, 12 June 2005, *A. Colelli* and *S. Costanzi* (UTV!); Montereale, 850 m, 18 July 2009, *L. Forti* (UTV!). **Campania**: Slopes of Vesuvio 570 m, 7 July 1983, *S. Jury* et al. (FI!); **Sardinia**: Oschiri, lago Coghinas in loc. S. Giorgio, 7 November 2006, *G. Calvia* (FI!). **Sicily**: Cefalù, sine die, Herb. *F. Tornabene* (CAT digital image!).